6. LABORATORY ANALYTICAL METHODS

The analytical methods implemented at the laboratory will comply with EPA-approved guidelines. The methods for conducting the tests of samples will be performed following specifications listed in the applicable EPA, PSEP, or National Council of the Paper Industry for Air and Stream Improvement (NCASI) methods. The analytical methods selected for use are listed in Table 5-1, along with the specified containers, preservatives, and holding times.

Sediment and marine biota analyses will be conducted in accordance with the guidelines presented in the *Recommended Protocols for Measuring Selected Environmental Variables in Puget Sound*, prepared by PSEP and associated updates for that document (PSEP, 1997). This guidance does not specify methods for the analyses of dioxins/furans, arsenic speciation, and resin acids. The dioxin/furan and arsenic speciation analyses will be performed in accordance with EPA Methods 1613B, 1632, and 1638 with detection limits consistent with appropriate sediment screening criteria and the requirements of the risk assessment portion of the Remedial Investigation. Likewise, resin acids will be performed using NCASI Method RA/FA-85.01 (modified).

6.1 CHEMICAL AND PHYSICAL TESTING

Chemical and physical testing of the sediment, marine biota, field blank, and equipment samples will be performed, where possible, in accordance with the methods contained in the PSEP guidance (with appropriate modifications). PSEP guidance has not been established for dioxin/furan, arsenic speciation, and resin acid analyses; therefore, these tests will be performed in accordance with EPA Method 1613B (for dioxins/furans), EPA Methods 1632 and 1638 (modified) (for arsenic speciation), and NCASI Method RA/FA-85.01 (modified) (for resin acids).

6.2 BIOASSAY TESTING

The purpose of the bioassay testing is to evaluate the degree and nature of potential toxicity to marine organisms resulting from potential sediment contamination near the project site. The testing is also intended to provide data for evaluation of chemical versus non-persistent compound-related (e.g., ammonia and sulfides) toxic effects.

6.2.1 Testing Program

SMS sediment biological testing will be performed at selected sediment locations and will consist of: 1) acute 10-day amphipod mortality, 2) acute 48-hour or 96-hour larval mortality/abnormality, and 3) chronic 20-day juvenile polychaete mortality and growth. The bioassay testing framework is designed to incorporate seasonal variability in species availability or sensitivity and varying grain size distributions. Sequim Bay sediments will likely be used as reference sediments for all toxicity tests. The tests will be conducted in accordance with protocols recommended by PSEP, as modified by the PSDDA program.

Treatment of Ammonia. The decay of organic matter may produce ammonia concentrations in sediment that are toxic to benthic organisms. Organic matter may be derived from natural as well as anthropogenic sources. Regardless of the source, the decay of organic matter in sediments may produce ammonia concentrations that are toxic to benthic organisms. Consequently, the biological testing program is designed to identify the presence and extent of toxicity, and to understand its cause (i.e., whether toxicity is derived from elevated contaminant concentrations or from organic enrichment).

Initially, bioassay test sediments will be tested for ammonia concentrations. If the ammonia exceeds thresholds noted in the bioassay protocols, the test sediments will be purged according to approved methods prior to conducting the toxicity tests. For the acute amphipod solid-phase test, an EPA-approved purging protocol (EPA, 1994) will be followed until porewater ammonia concentrations are reduced to below 30 mg/l. The purging protocol used for the acute amphipod test will also be used for the larval and polychaete tests. Ammonia thresholds are 30 mg/L for the larval test and 20 mg/L for the polychaete test. For the larval test, in addition to the standard PSEP tests, test solutions will be aerated and reference toxicant exposures will be conducted with ammonia (Ecology, 1995). At the initiation of the bioassays, sediment porewaters will be analyzed for ammonia. During the tests, overlying waters will be monitored for ammonia at the end of the test; sediment porewater will be retested. Concurrently, laboratory reference toxicant experiments will be performed on the bioassay test organisms to establish the toxicity of ammonia that will likely contribute to adverse effects in test organisms and possible failures of test criteria. The test will be conducted in a manner similar to the standard reference

toxicant test, which is based on a dilution series. An LC50 or EC50 will be calculated based on concentrations of ammonia in sediment interstitial water for the amphipod tests and on overlying water ammonia concentrations for the larval tests. The results of the ammonia positive control tests (e.g., the LC50s) will be compared with the porewater (amphipod and polychaete tests) and overlying water (larval test) ammonia results and used in the interpretation of the test results.

6.2.2 Amphipod Bioassay

In the acute amphipod bioassay, short-term, adverse effects of potentially contaminated sediment will be evaluated by measuring survival in adult phoxocephalid amphipods (e.g., *Rhepoxynius abronius, Ampelisca abdita*). Amphipods will be exposed to the surface sediments near the former Rayonier Mill Site and reference areas (e.g., Sequim Bay) for a 10-day period. The test will be performed according to the procedures and QA/QC performance standards following PSEP (1995), with survival as the endpoint. In addition to the control and reference samples required by PSEP, an ammonia positive control test will be conducted. The purpose of conducting an ammonia positive control test is to determine the toxicity of ammonia to the test organisms being used in this study.

6.2.3 Larval Bioassay

In general, any one of five larval species can be used for this test. Selection of an appropriate test species is dependent on the seasonal availability of adult organisms that can produce viable gametes. However, experience with past larval tests indicates that bivalve larva of *Mytilus* sp. or echinoderm larvae of the sanddollar *Dendraster excentricus* provide the most consistent and reliable results. Consequently, for this program, the laboratory will select the best available of these species during the week preceding delivery of the initial sediment samples.

The acute larval bioassay is primarily an indicator of the relative toxicity in sediment samples because larvae (e.g., *Crassostrea gigas, Mytilus edulis, Dendraster excentricus, Strongylocentrotus purpuratus*) normally reside in the water column and are not intimately associated with sediments. Three endpoints are measured after a 48-hour exposure period: mortality, abnormal development, and combined mortality/abnormality. Test protocols and quality assurance/quality control (QA/QC) performance standards will be in accordance with PSEP (1995). In addition to the control and reference samples required by PSEP, an

ammonia positive control test will be conducted. The purpose of conducting an ammonia positive control test is to determine the toxicity of ammonia to the test organisms being used in this study.

6.2.4 Juvenile Polychaete Bioassay

The chronic bioassay is used to characterize chronic, sublethal toxicity of potentially contaminated marine sediments based on survival and growth of *Neanthes arenaceodentata.* The test will be performed according to the procedures and QA/QC performance standards following PSEP (1995). Juvenile worms are exposed to test sediments for 20 days. Water is replaced periodically and the worms are fed during the test. At the end of the test, surviving worms are enumerated and weighed to determine their biomass. In addition to the control and reference samples required by PSEP, an ammonia positive control test will be conducted if ammonia in thresholds are exceeded. The purpose of the ammonia positive control is to determine the toxicity of ammonia to test organisms used on this study.